

CHROMOSOME STUDIES

IN

NICANDRA PHYSALOIDES

BY

E. K. JANAKI-AMMAL

DEPARTMENT OF BOTANY — UNIVERSITY OF MICHIGAN (U. S. A.)

(Reprinted from « La Cellule », volume XLI, fascicule 2, 1932)

Paper received October 11, 1931

Ann Arbor

LIERRE

TYP. DE JOSEPH VAN IN & C^{ie}
Grand'place, 37



LOUVAIN

A. UYSTPRUYST, ÉDITEUR
rue de la Monnaie

LA CELLULE

Recueil de travaux originaux

de Cytologie, de Biologie et d'Histologie générale

PUBLIÉ AVEC LE CONCOURS DE LA FONDATION UNIVERSITAIRE DE BELGIQUE

Cette Revue paraît par fascicules d'importance variable. Deux à quatre fascicules constituent un tome — généralement annuel — de 450 pages in 4°, avec 20-35 planches en double page (noires ou en couleurs) et des figures dans le texte.

Prix de l'abonnement (par volume) : 10 \$ ou 350 frs. belges, ce montant comportant les frais d'envoi. Il est abaissé à 6 \$ pour les pays à change déprécié (France, Italie, Pologne, Roumanie, Yougoslavie, Tchécoslovaquie) et à 175 frs. belges (5 \$) pour la Belgique. Pour les libraires, ces réductions sont subordonnées à la désignation des destinataires.

Les abonnements sont reçus par l'Éditeur : **A. UYSTPRUYST, Librairie Universitaire, rue de la Monnaie, Louvain.**

* * *

Les auteurs sont priés de n'envoyer que des mémoires *dactylographiés* ou, au moins, très lisiblement écrits. Les dessins destinés aux *planches hors-texte* seront faits à l'encre de chine ou au crayon-carbone. S'ils doivent être reproduits en *lithographie*, ils seront accompagnés d'un calque indiquant leurs positions respectives (justification : 31 X 23 cm. pour les planches en double page; 15 1/2 X 23 cm. pour les planches simples). S'ils doivent faire l'objet d'une reproduction *photographique* (collotypie, simili-gravure, zincogravure), ils seront collés en place sur carton *fort*, et le n° de chaque figure sera clairement indiqué à l'encre de chine. Les dessins destinés à figurer *dans le texte* seront faits, de préférence, *au trait* et à l'encre de chine.

Soixante-quinze exemplaires de leurs travaux sont remis gratuitement aux auteurs. Les exemplaires supplémentaires sont fournis au tarif suivant : Par feuille de 8 pages; 25 exemplaires : frs. 16—; 50 ex. : frs. 24—; 75 ex. : frs. 32—; 100 ex. : frs. 40—; chaque centaine en plus : frs. 30—. En sus : couverture (fr. 0,27 par exemplaire), frais de tirage des planches (suivant format et procédé de reproduction) et port.

Les manuscrits et toute demande concernant *la Rédaction et les Échanges* doivent être adressés à **M^r P. MARTENS**, professeur à l'Université, rue Marie-Thérèse, 23, Louvain.

Les Directeurs :

G. GILSON & V. GRÉGOIRE

PROFESSEURS A L'UNIVERSITÉ DE LOUVAIN.

Sommaire du fascicule 2 :

- E. K. JANAKI-AMMAL (Madras, Indes) : Chromosome studies in *Nicandra physaloides* (2 planches).
R. MOSSERAY (Louvain) : Influence du zinc sur les *Aspergillus* du groupe *niger* et sur quelques autres (1 planche).
P. MARTENS & R. CHAMBERS (Louvain) : Études de microdissection. V. Les poils staminaux de *Tradescantia* (1 planche).
L. M. SCHEUBER (Wisconsin, U. S. A.) : A cytological study of *Timmia cucullata* (2 planches).
HSU-LIANG (Péking) : Structure of somatic chromosomes in *Lilium tigrinum* (1 pl.).
A. CONET (Louvain) : Le cycle évolutif du *Plistophora chironomi* (1 planche).

CHROMOSOME STUDIES

IN

NICANDRA PHYSALOIDES

BY

E. K. JANAKI-AMMAL

DEPARTMENT OF BOTANY — UNIVERSITY OF MICHIGAN (U. S. A.)

(Reprinted from « La Cellule », volume XLI, fascicule 2, 1932)

Paper received October 11, 1931

A dissertation submitted in partial fulfillment of the requirements
for the degree of Doctor of Philosophy in the University of Michigan.



CHROMOSOME STUDIES

IN

NICANDRA PHYSALOIDES*

INTRODUCTION.

Nicandra physaloides (L.) GAERTN. is an annual reputed to be of Peruvian origin. It is a monotypic genus now widely distributed both in the New and Old World. According to the classification of ENGLER and PRANTL, *Nicandra* is placed in a separate tribe of the Solanaceae on the basis of the 3-5 celled ovary which is exceptional in this group of plants where the ovaries in most of the genera have 2 cells. Though considerable attention has been given to related genera, the only cytological investigation on *Nicandra* is the report of a chromosome count published by VILMORIN and SIMONET (1927). They gave the haploid number as 10, which this paper confirms.

The purpose of this investigation was to make a detailed study of meiosis and of the chromosomes in comparison with those of related genera. It was hoped that some light might be thrown on the problem of the affinities of this genus.

To Professor BARTLETT and Dr BLANCHARD I wish to express my great appreciation of the assistance given by the Botanical Garden in the progress of the studies.

For helpful interest and criticism throughout the course of this work I am indebted to Dr B. M. DAVIS under whose direction the investigation has been conducted.

MATERIAL AND METHODS.

Seeds of *Nicandra physaloides* were collected by the writer in 1927 on the Wynaad Plateau, South India. Plants were raised from these seeds in 1929 at the Botanical Gardens of the University of Michigan and in 1930 a second generation was grown. In 1930 plants were also obtained from seeds of an American race of *Nicandra physaloides* taken from a herbarium specimen at the University of Michigan. This material was collected in Tennessee by C. O. ERLANSON in 1926. Plants were grown both in the green house and in the field and material for study was collected from both cultures.

The Indian material belonged to the variety *immaculata* and the American material to the variety *typica* described by DAHLGREN (1924), in his genetical

(*) Papers from the Department of Botany, University of Michigan, N° 358.

studies on these two varieties. The two varieties are distinguished by the presence of five blue spots at the base of the corolla in *typica* and their absence in *immaculata*. No cytological difference could be found between the two varieties.

Nicandra physaloides is very susceptible to environmental changes and easily succumbs to drought or frost. In poor dry soil the plants are scarcely a few inches in height whereas, with abundance of water and shade, they grow to a height of 6 ft. and have large leaves.

Anthers were fixed in a number of fluids. The following modification of BOUVIN's solution gave the best results.

SOLUTION A :		SOLUTION B :	
Saturated solution of Picric acid	50 cc.	Saturated solution of Picric acid	25 cc.
Glacial acetic acid	5 cc.	Commercial Formalin	25 cc.
Chromic acid	1 gm.	Urea crystals	1 gm.

Equal parts of solution A and B were mixed at the time of fixation. Buds were allowed to remain in the fluid from 4-6 hours and were then run rapidly through alcohols beginning with 35 % to 85 % where they were left until all trace of picric acid was removed by constant change of alcohol.

Material fixed in strong FLEMMING's solution and in acetic alcohol in the ratio of 1 part acetic acid : 3 parts absolute alcohol also gave good results. KARPECHENKO's fluid (1924) and the combination of CARNOY's acetic-alcohol-chloroform with NAWASHIN's fluid as used by Takeshinge MAEDA (1928) proved very satisfactory for the study of chromosome morphology even though it produced fibrils in the cytoplasm. McCLINTOCK's method (1929) of staining with acetocarmine after fixation in acetic alcohol was useful in preliminary studies, to enable buds to be picked at the right stage. Anthers were fixed at all times of the day; for the study of meiosis, fixations from 11 : 30 AM to 1 : 30 PM proved most satisfactory.

Nicandra physaloides was found to offer unusual advantages for the study of progressive stages in meiosis because of the slight difference in age found among the anthers of the same flower. A still finer gradation in development existed in the pollen mother cells of the same anther. This phenomenon has been found in other Solanaceous plants, notably in tomatoes by LESLEY (1926), in diploid *Nicotiana tabacum* by GOODSPEED (1923) and in species of *Solanum* by the writer. In the developing anthers the youngest stages in the development of the microsporocytes were found in the basal region and later stages in the apical regions of the same locus. The microsporocytes in the loculi of one anther were as a rule approximately at the same stages of development but sometimes one locus was slightly in advance of the other.

The following were a few of the serialations found in anthers taken from the same bud : anther 1, spireme to diakinesis; anther 2, heterotypic metaphase to homoeotypic metaphase; anther 3, heterotypic metaphase to telophase of homoeotypic division; anther 4, heterotypic metaphase to tetrad.

Sections were cut from 3 μ . to 18 μ . thus permitting a study of complete cells, which is necessary for an accurate understanding of nuclear structure and chromosome configuration. Most of the sections were stained with HEIDENHAIN's iron alum haematoxylin. Iodine Gentian Violet as used by NEWTON and DARLINGTON (1929) and FLEMMING's triple stain also gave good results. TAYLOR's method of smear fixation (1924) was tried in 1930. It did not prove so satisfactory as with monocotyledonous material, but it was helpful in confirming interpretations made through studies by the paraffin method.

I. THE ORGANIZATION OF CHROMOSOMES IN THE RESTING NUCLEI OF SOMATIC CELLS.

The structure of a «typical» nucleus in the resting stage has been generally described as a more or less continuous network imbedded in the karyolymph or nuclear sap. *Allium*, *Vicia Faba* and *Lilium* have provided standard material for the study of this type of resting nucleus.

The resting nucleus in *Nicandra* however, presents a very different type of structure from the above. In the cells of the anther wall, the nucellus of the developing ovule and in other parts of the bud where active cell division has ended, the nuclei contain deeply staining bodies associated in pairs (FIG. 1), and evenly distributed at the periphery. They are connected with one another by very fine linin strands. Considerable variation has been found both in the size of these bodies and the manner of their association, FIG. 10. One pair much longer than the others can be easily identified in each nucleus, FIG. 1, *a*. The two bodies of each pair may be found apparently united at one or both ends, thus forming V's and O's, or they lie side by side without contact. The latter arrangement is more general among the smaller pairs. In configuration they resemble the gemini found in the diakinesis stage of meiosis in the pollen mother cells. In material suitably fixed, a single chromatic spiral band corresponding to the chromonemata described by BONNEVIE (1908), is found running lengthwise through each body. This is surrounded by a more lightly staining cylinder which forms the boundary of each body.

The number of such pairs found in each somatic nucleus excepting those of the fully developed tapetal cells is 40, which number corresponds to the haploid count for *Nicandra physaloides*. Because of their close resemblance both in structure and form to the bivalent chromosomes of meiosis, I believe these paired bodies are the chromosomes themselves which in *Nicandra* are associated in homologous pairs in these somatic cells and which undergo little or no change during rest.

DE LITARDIÈRE (1921, *b*) has reported that the small chromosomes found in *Salvinia* and *Azolla* remain clearly visible through interphase in somatic cells. OVERTON (1909) has described as «prochromosomes» paired bodies found by him in the resting somatic cells of *Thalictrum purpurascens*, *Calycanthus floridus* and *Richardia africana*. In the root tips of *Calycanthus* he found them as large as

ordinary somatic chromosomes and having the same form. For this reason OVERTON thought that they must represent the greater part of the chromosomes. ROSENBERG (1904, 1909) observed paired chromosomes in the somatic cells of *Capsella* and *Crepis virens* and STRASEBURGER (1907) maintained that the parental chromosomes become closely associated while retaining their individuality in somatic cells of *Pisum sativum*.

II. SOMATIC MITOSIS.

To understand clearly the nature of the chromosome pairs found in the nuclei of resting cells and to connect their history with the events during the two meiotic divisions of the microsporocytes, it is essential that vegetative mitosis in the sporophyte be understood.

These may be readily studied in root tips, in actively dividing cells of the anther or in the archesporium just before the differentiation of the microsporocytes. No differences in the mode of division were found in cells from these three regions. However, since the last somatic division in the archesporium is of particular interest because of its close relationship to the nucleus of the microsporocyte, greater attention was paid to this mitosis.

A cell about to divide is clearly distinguished from those at rest by the increased density of its cytoplasm which consequently stains deeply. The nucleus and nucleolus are slightly larger than those of other somatic cells. The approach of mitosis is first indicated by a gradual elongation of the 10 pairs of chromosomes lying at the periphery of the nucleus. The pairs approach one another as the fine linin thread that connects them shorten with the elongation of the chromosomes. During elongation the mates that lie parallel to each other separate, FIG. 3. This finally results in the formation of a system of coiled threads, in which it is impossible to recognize the homologous chromosomes of each pair. The staining quality of the threads increase considerably at this stage and it is very difficult to recognize differentiation into chromatic and achromatic regions in material stained with iron alum haematoxylin. However, in root tips fixed in strong FLEMMING'S and stained with Iodine Gentian violet a double thread has been observed, FIG. 4, which suggests that the lengthwise split occurs at this stage or earlier to form daughter chromosomes.

The coiled threads next thicken and simultaneously grow shorter. This brings about a clear definition of the 20 chromosomes, FIG. 5, which have the appearance of bent rods. Their lengthwise division is quite evident now. The nucleolus which by this time has largely lost its staining capacity suddenly disappears and at the same time the nuclear membrane breaks down and fibrillae appear in the nuclear cavity. The split chromosomes become massed in the middle of the cell, and arrange themselves to form the characteristic metaphase plate, as shown in FIG. 6. The bi-polar spindle is very sharply outlined with the spindle fibres attached to the middle of each daughter chromosome, which become V shaped. A transverse section through the equatorial region

shows 20 chromosomes, FIG. 7, on the metaphase plate. The daughter chromosomes proceed to the pole with great uniformity, FIG. 8. It is not very difficult to recognize individual chromosomes at telophase but this is facilitated when the chromosomes begin to shorten. Areas of nuclear sap appear to form around each chromosome and these seem gradually to coalesce. The nuclear membrane is formed where the nuclear sap makes contact with the cytoplasm, FIG. 9. At this stage it is possible to observe chromosomes coming together in pairs, FIG. 9, to give the condition present in the resting nucleus, FIG. 1.

III. RESTING NUCLEI OF THE MICROSPOROCTES.

In *Nicandra physaloides* a single layer of archesporial cells is differentiated very early in the development of the anther. These cells by progressive somatic divisions give rise to two rows of microsporocytes. The last somatic division of the archesporial cells has been very closely followed in the study of mitosis and found to be similar to other somatic divisions.

The young microsporocytes, FIG. 10, have very dense protoplasm and the nuclei are somewhat larger than those of other somatic cells. The 10 pairs of chromosomes are very clear and they present the structure characteristic of somatic cells. There is generally a single deeply staining nucleolus but occasionally two smaller nucleoli have been observed.

The period of so called «rest» between the last somatic mitosis and the prophase of the meiotic divisions covers in *Nicandra*, at least 3-4 days, judging from the comparative study of very young anthers. Figures 10-13 represent serially the changes within the nucleus during this period. The chromosomes present a more irregular outline due to the elongation of the chromonemata from both ends of the chromosome body. This leads to their lengthening, FIG. 11, and the process of elongation continues as seen in fig. 12.

BEER (1913) noted similar paired arrangement of chromatic bodies in the microsporocytes of *Equisetum arvense* which he considered as chance phenomena depending upon «natural or artificial tensions». Such an interpretation is out of the question in *Nicandra* where the gradual evolution of the thread system can be traced.

The final result of all this activity in the «resting nucleus» is a network of fine threads, FIG. 13, which is the leptonema of the prophase of meiosis. Individual zigzag threads believed to correspond to the spiral chromonemata found in somatic cells, FIG. 2, can be often distinguished where the fixation is satisfactory.

The literature of meiosis contains many references to prochromosomes, «gamosomen» and other chromatic bodies occurring in the resting pollen mother cells. MIYAKE (1905) noticed them in *Galtonia*; YAMANOUCHI (1906) in *Poly-siphonia*; OVERTON (1905, 1909) in *Thalictrum*, *Calycanthus* and *Richardia*;

LAIBACH (1907) in *Sisymbrium*, *Brassica*, *Stenophragma*, *Alyssum*, *Iberis* and *Lunaria*; ROSENBERG (1904, 1907, 1909) in *Zostera*, *Capsella*, *Hieracium*, *Drosera* and *Crepis*; LUNDEGÅRDH (1909) in *Calendula*; SCHAFFNER (1909) in *Agave*; CAMPIN (1925) in *Nolana*; WEIER (1927) in *Oenothera*. Some of these authors regard the structures as early stages of chromosome formation and other authors interpret them as late stages in the expansion of the chromosome prior to the formation of a reticulum. In *Nicandra* the latter interpretation is unquestionably correct. The writer believes that in a number of cases where prochromosomes have been noted in the resting microsporocytes, careful studies would have determined their origin from chromosomes of the previous somatic telophase.

IV. MEIOSIS IN THE MICROSPOROCYTES.

1. Heterotypic Prophase.

a) Presynizesis.

At the close of the resting period and just before synizesis, the nucleus of the microsporocyte is filled with a close system of delicate threads, which give the appearance of a reticulum, FIG. 13. It is impossible to say with certainty whether such a system is formed from a single thread or from a number of separate strands. As far as the writer can judge from the study of sections in which whole nuclei were observed there are no free ends but it is doubtful whether free ends could be seen in fixed material. The threads also show no more parallelism than could be expected by chance associations in so close a mass. An increasing thickness of the threads which become more granular in structure and stain more deeply, marks the beginning of a change in this reticulum.

b) Synizesis.

The first indication of the approach of synizesis is the gradual contraction of the thread system away from the nuclear membrane so as to leave a clear space, FIG. 14. This becomes intensified by an expansion of the nuclear area. The mass of threads takes a position generally on one side of the nucleus, with the nucleolus completely surrounded by the coils. As synizesis progresses the threads thicken and the nucleolus stains very poorly with basic dyes. In some cells small masses of chromatic material resembling nucleoli may also be found among the threads.

During synizesis the cell appears to be very sensitive to osmotic disturbance. Cytomixis is sometimes observed. Many investigators believe that synizesis is an artefact due to imperfect fixation. If this is so, cytomixis may be considered an extreme effect of such disturbance. According to BĚLAŘ (1922) during prophase two processes take place side by side, first, a reduction in the viscosity of the nuclear ground substance and, second, an increase in the viscosity of the chromosomes. It is possible that synizesis occurs at the time when the

viscosity of the supporting medium has been reduced without the rigidity of the chromosomes being sufficiently increased to enable them to stand the effects of the fixing fluids. DARLINGTON (1929) gave this explanation for synizesis in hyacinths. In his investigation on tulips, where he used smear preparation, he found no synizesis (NEWTON & DARLINGTON, 1929). NEWTON (1927) using the same smear technique stated «that a certain amount of contraction towards one side of the nucleus actually exists». Such contraction has been described by Miss SARGANT (1896) in living cells of the Lily, and by KATO (1930) also in living cells of *Rhoeo discolor* and *Lilium speciosum*. TAYLOR (1922) reported no synizesis in *Gasteria*. In *Nicandra* synizesis was observed both in smears and in paraffin material though it was less pronounced in the former. This condition persists for a long time as shown by its frequency in fixed material of *Nicandra* and its prevalence simultaneously in all anthers of the same bud.

c) *Pachytene*.

Recovery from the synizesis artefact takes place slowly, FIG. 15. The pachytène thread finally becomes arranged in loops along the periphery of the nucleus. It is much thicker than the threads of leptonema since it consists of paired threads (compare FIG. 15 with FIG. 13). KAUFMANN (1926), KUWADA and SUGIMOTO (1926) and SAKAMURA (1926) figure similar threads in *Tradescantia* and KUWADA (1926, 1927) in *Vicia Faba*. MAEDA (1930) finds a rope-like twist in the pachytène threads of the sweet pea. BABCOCK and CLAUSEN (1929) report the same structure in *Crepis*. SHINKE (1930) using aceto-carminic methods has demonstrated that this is the nature of the thread in many plants. The pachytène threads finally fill the cavity of the nucleus with many loops. The appearance of a continuous thread is doubtless due to contraction, FIG. 16. A continuous spireme has been reported for a number of the *Solanaceae*. O'NEAL (1920) finds it in *Datura*, LESLEY (1926) in *Tomato*, GOODSPEED (1923) and CHIPMAN and GOODSPEED (1927) in *Nicotiana*.

d) *Diplotene* and *Diakinesis*.

Condensation of the pachytène thread progresses gradually without the conspicuous looping or folding observed in the so-called «second contraction» described for such material as *Lilium*, *Oenothera* and *Lathyrus*. The next stage to be observed is an early diplotène. Ten bivalents can be distinguished each composed of two parallel chromosomes united at one or both ends or interstitially. Where two univalents cross each other, one or two nodes or «cross points» are found. These points of contact can be shown to be chiasmata by their later behaviour and will be discussed later. The rope-like twist of the two strands in each thread observed earlier, persists so that the bivalents consist of four strands and represent a tetrad of chromatids. Rapid contraction and thickening of the bivalents proceeds, FIGS. 17 and 18. They are of different sizes and forms, as will be described in detail later. One large bivalent, FIG. 18, L_2 , is particularly conspicuous and appears to be the large pair of chromosomes found in the resting somatic cell, FIG. 1, a. The quadrivalent nature of

the bivalents is less evident at this time because of the increased condensation of the chromosomes and their greater affinity for stains. The pairs take a peripheral position in the nuclear cavity.

2. Heterotypic Metaphase.

The nucleolus which during diakinesis occupied a position on one side of the nucleus, suddenly disappears. In a few cells a slight budding of the nucleolus could be observed before its disorganisation. Simultaneously with the disappearance of the nucleolus, the nuclear membrane breaks down, FIG. 19, and when this occurs the chromosome pairs now very much condensed, become grouped in a close mass in the centre of the cell, FIG. 20. Some of the bivalents are generally found to be joined one to another by delicate threads.

Spindle fibres appear both in the cytoplasm and in the nuclear cavity surrounding the group of chromosomes. They later become attached to the middle region of each chromosome and an irregular multipolar spindle can be seen, FIG. 21. This multipolar condition gradually changes into a typical bipolar spindle characteristic of metaphase, FIG. 22. The chromosomes are arranged on the metaphase plate with two or three bivalents frequently in the centre of the group and the remaining ones forming a circle around them. The degree of condensation and the clearness with which the configuration of the bivalents can be observed depends largely on the type of fixative employed. This configuration, FIG. 22, which will be described in Section VI was found to be particularly well shown in material fixed with acetic alcohol in the ratio of 1 acetic acid : 3 absolute alcohol. The split which occurred during prophase is not evident during metaphase.

3. Heterotypic Anaphase and Telophase.

The separated univalents, FIG. 23, pass to both poles in groups of 10 as **V** shaped structures with their apices pointing to the poles. Polar views during anaphase show the lengthwise split of prophase, FIG. 24. When the chromosomes arrive at the poles they form a crowded group and it is only with difficulty that individual chromosomes can be recognized. The chromosomes slowly separate from one another, apparently with the secretion of karyolymph between them and a nuclear membrane is formed where the nuclear sap comes into contact with the cytoplasm, FIG. 25. One or more nucleoli appear in the daughter nuclei and the spindle fibres disappear.

4. Interkinesis.

A period of rest intervenes between the first and second meiotic divisions, during which the daughter nuclei occupy a large part of the pollen mother cell. The 10 split chromosomes undergo no marked change during interkinesis. The halves lie side by side or are joined at some point forming **V**'s and **X**'s. The large pair of chromosomes found in somatic cells, FIG. 1, *a*, and at diakinesis, FIG. 19, *L*₂, can again be recognized.

5. Homeotypic Division.

In preparation for the homeotypic division, the nuclear membranes of the daughter nuclei break down simultaneously with the disappearance of the nucleoli, and the 10 split chromosomes, some of which are joined to one another by delicate fibres, are assembled as in FIG. 26.

The multipolar spindle formed about each group of chromosomes soon becomes bipolar, FIG. 27. The two spindles in each microsporocyte may lie in the same plane or at right angles to one another. The daughter chromosomes when arranged on the equatorial plate are very much compressed and almost globular, FIG. 27.

The stages of anaphase, FIGS. 28 and 29, are passed through very rapidly. Except for occasional lagging as observed in some cells, FIG. 29, the movement of the chromosomes to the poles is simultaneous. Division of the chromosomes has not been observed during anaphase. On reaching the poles the chromosomes form a close mass. After the formation of the grand daughter nuclei, each chromosome is found to be divided lengthwise, FIG. 30. At this stage deeply staining granules are present in the cytoplasm in the region of the fibres that are formed between the nuclei, FIG. 31.

6. Cytokinesis.

The four daughter nuclei in the microsporocyte are connected with one another by fibres which persist until cell division is almost complete, FIG. 31. The pollen tetrad is formed in *Nicandra* by means of furrows or invaginations of the cytoplasm. These proceed from the periphery towards the centre, and thus divide the microsporocyte simultaneously into four tetrahedral daughter cells with the nuclei equidistant from the centre. The mother cell wall, which has now increased in thickness very considerably is found to follow the membrane of the invaginating furrow. When cytokinesis is complete, the four pollen grains lie within the walls of the original microsporocyte.

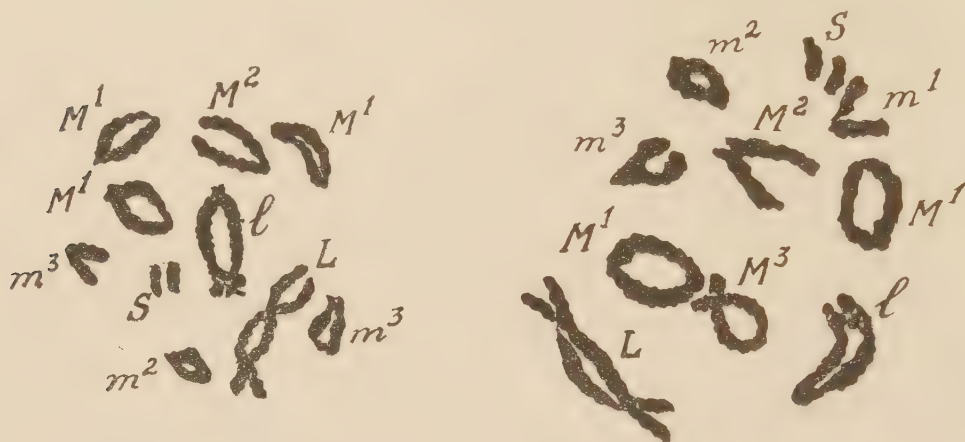
The chromatic granules seen in the cytoplasm during the telophase of the homeotypic division increase in number and size during cytokinesis. The chromosomes at this stage are slender rod-shaped bodies in which the split may often be seen, FIG. 31. There is a single large or several smaller nucleoli in each nucleus. The microspores become more ellipsoidal in shape and are finally set free in the pollen sac by the disorganisation of the mother cell wall.

Quadripartition by furrowing and not by the formation of «cell plates» as was believed to occur by STRASBURGER and others, is now accepted as the general method of tetrad formation in plants. The extensive studies of C. H. FARR (1916, 1918, 1922) on the microsporocytes of *Nicotiana*, *Magnolia*, *Sisyrinchium* and *Nelumbo* led the investigations in the field. His conclusion in general have been sustained by the work of W. K. FARR (1920) in *Cobaea scandens*, CASTELLER (1925, 1926) in *Melilotus* and *Curcubita*, CAMPIN (1925) in *Nolana*,

WEINSTEIN (1926) in *Phaseolus*, GATES (1927) in *Lathraea*, HEIMLICH (1928) in *Cucumis*, KULKARNI (1929) in *Oenothera*, to which my observations on *Nicandra* contribute support.

V. THE CONFIGURATION OF BIVALENTS IN THE HETEROTYPIC DIVISION.

The chromosomes of *Nicandra physaloides* are found to be of different sizes. One pair longer than the others has been noted both in the somatic cells, FIG. 1, and in microsporocytes, FIG. 18. Examination of several hundreds of pollen mother cells at diakinesis and the comparative study of camera lucida drawings of many microsporocytes in which all of the 10 bivalents were present, as in text fig. 1 and 2, has led the writer to believe that at least 5 different lengths are represented by the chromosomes in *Nicandra*. The chromosome

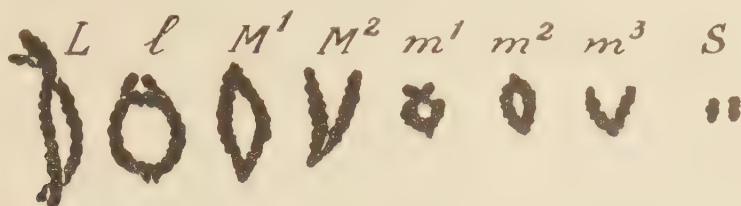


TEXT FIG. 1 AND 2. Complete set of bivalents from two microsporocytes at diakinesis. The letters indicate the types of chromosomes lengths.

complex approximates to the formula $L_2 + l_2 + 4M_2 + 3m_2 + S_2$ where L is the longest chromosome, l the second longest, M the medium sized, m the secondary medium and S the shortest chromosome. The configurations of the bivalents not being similar, it is possible that certain gradations between the chromosomes may be exaggerated or reduced according to the form taken. Thus a bivalent in the form of a ring (that is, having 2 points of attachment or 2 chiasmata) appears to be much shorter than one which is V shaped (having only one attachment or chiasma) even though the two have univalents similar in length. The formula presented is based wholly on observations upon bivalents in the heterotypic prophase.

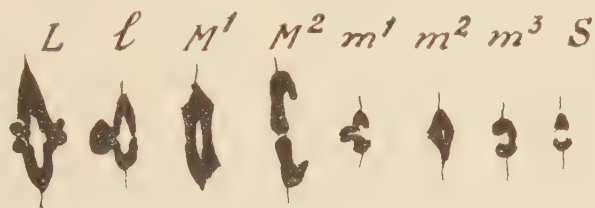
Text fig. 3 gives the configuration of the types of bivalent chromosomes during diakinesis and text fig. 4 their corresponding forms during metaphase. The points of contact between the chromosomes in the bivalents seem to be analogous to the chiasmata observed in many plants and animals. DARLINGTON's interpretation of end to end association of chromosomes as terminal chiasmata has been followed (DARLINGTON, 1929). In the case of bivalents

L, *l* and *m* which have interstitial chiasmata that are not completely «terminalized», the nodes are found to persist up to metaphase.



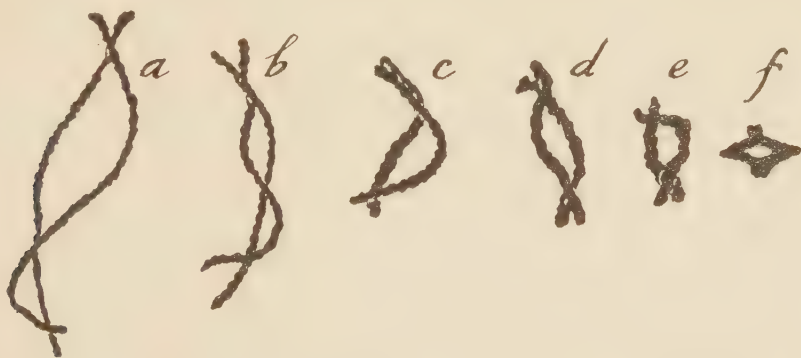
TEXT FIG. 3. Forms of the *L*, *l*, *M*, *m* and *S* bivalents during late diakinesis.

Observations in *Nicandra* show that the configuration of the bivalents do not differ greatly between earlier and later stages in diakinesis. This is especially the case with the short pairs of chromosomes. With respect to length, there are five types of chromosomes to be considered as follows: 1. In the longest



TEXT FIG. 4. Side views of the *L*, *l*, *M*, *m* and *S* bivalents during metaphase.

pair, *L*, TEXT FIG. 1 and 2, the component chromosomes have two interstitial chiasmata at some distance from the ends and in very early stages of diakinesis there are occasionally three interstitial chiasmata as shown in text fig. 5, *a* and *b*. In later diakinesis the paired chromosomes condense and become

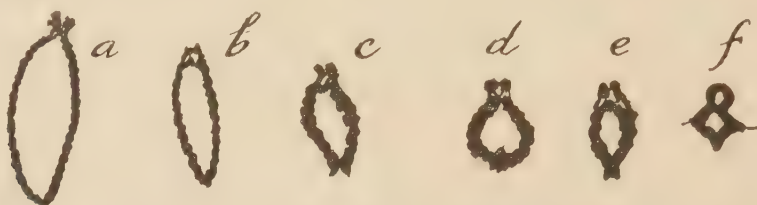


TEXT FIG. 5. Stages in the condensation of the longest or *L* bivalent; *a* and *b* early diplotene; *c*, *d*, *e*, diakinesis; *f*, metaphase.

widely separated from each other and thus form a loop with two free arms beyond the 2 subterminal interstitial chiasmata, TEXT FIG. 5, *c*, *d*, *e*. During metaphase where the spindle fibres are attached to the middle region of each univalent, the loop is drawn into a ring form, along the direction of the fibres, but the subterminal interstitial chiasmata persist on both sides as shown in

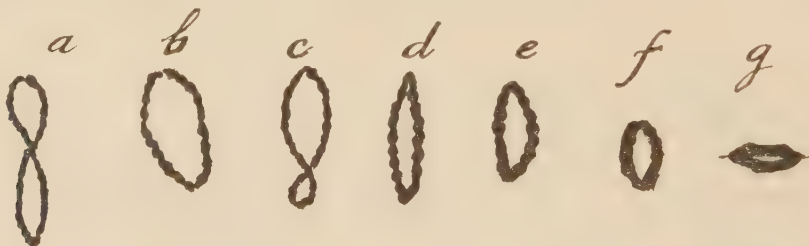
text fig. 4, *L*, and text fig. 5, *f*. Successive stages in the condensation of this pair of chromosomes are shown in text fig. 5.

2. In the next longest pair, «*l*», the chromosomes at early diakinesis are usually joined at one end by a terminal chiasma and at the other by a subterminal interstitial chiasma, TEXT FIG. 1, 2, 3, *l*. As contraction proceeds the component chromatids are separated at this end and the four stranded nature of the bivalent is evident. During metaphase what seems to be one or two terminal and one interstitial chiasmata have been observed. The persisting interstitial chiasma forms a «hump» in the metaphase configuration, TEXT FIG. 4, *l*, and 6, *f*. The successive stages in the condensation of this chromosome are shown in text fig. 6.

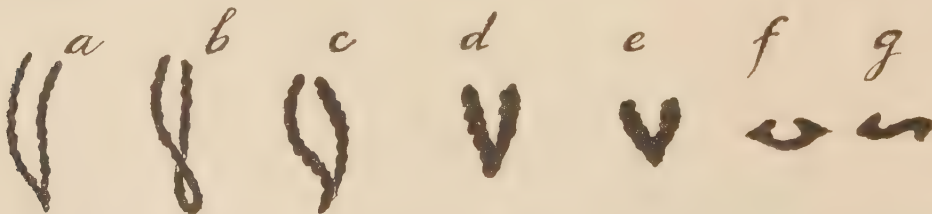


TEXT FIG. 6. Stages in the condensation of the second longest or *l* bivalent from diplotene to metaphase; *a*, late diplotene; *b*, early diakinesis; *c*, *d*, *e*, later diakinesis; *f*, metaphase

3. In the longer of the medium sized pairs, the «*M*» pairs of which there are four, the chromosomes are attached to each other at both ends by terminal chiasmata to form a ring, TEXT FIG. 1, 2, 3, *M*¹, or there is only a single terminal chiasma which gives a **V**, TEXT FIG. 1, 2, 3, *M*². Rarely one interstitial and one terminal chiasma may be found in this pair (see text fig. 2, *M*³). Text figures 7 and 8 show the configurations in the longer of the median sized pairs from late diplotene to metaphase.



TEXT FIG. 7. Stages in the condensation of an «*M*» bivalent with two terminal chiasmata from diplotene to metaphase; *a*, diplotene; *b*, *c*, and *d* early diakinesis; *e* and *f*, late diakinesis; *g*, metaphase.



TEXT FIG. 8. Stages in the condensation of an «*M*» bivalent with a single terminal attachment from diplotene to metaphase; *a* and *b*, diplotene; *c*, early diakinesis; *d* and *e*, late diakinesis; *f*, and *g*, metaphase.

4. In the case of the smaller medium sized pairs, «*m*» of which there are three, the chromosomes are attached to each other at one or both ends by terminal or subterminal chiasmata, TEXT FIG. 1, 2, 3, m^1 , m^2 , m^3 . The configurations are similar to those in the «*M*» pair. When the univalents are attached at only one end there may rarely be a subterminal interstitial chiasma at the other end, TEXT FIG. 3, m^1 , which sometimes persists until metaphase, giving a «hump» in the rings, TEXT FIG. 4, m^1 .

5. In the shortest pair «*S*» the component chromosomes are associated side by side, TEXT FIG. 1, 2, 3, *S*. A thin fibre generally connects the two univalents at one or both ends. Rarely these chromosomes have been found to form **X** and **V** configurations. During metaphase the fibre connection is clearly visible, TEXT FIG. 4, *S*. The small size of the bivalent suggests the possibility that it may have arisen through fragmentation of a larger chromosome.

VI. DISCUSSION.

Chromosome Structure.

BARANETZKY as early as 1880 noted a spiral structure of the chromonemata in *Tradescantia*, and again BONNEVIE (1908, 1911) described it in *Ascaris* and *Allium*. With the development of cytological technique and especially the smear method, as described by TAYLOR (1924), spiral chromonemata have been frequently described both in fixed and living material (references given in Section V), and the condition is now accepted as a feature of chromosome structure in many plants. There is however considerable difference of opinion with respect to the nature of the spiral at different stages in the mitotic and meiotic cycle. BONNEVIE postulates a single chromonema at anaphase and telophase. This is also the view held by MARTENS (1922, 1925) in his work on *Paris quadrifolia* and *Listera ovata* where he finds the chromosome is single from anaphase to early prophase but becomes double during the middle and late prophase. KAUFMANN (1925, 1926) working on *Tradescantia* and *Podophyllum* finds the chromonemata are double during telophase of mitosis. He considers the single thread found by BONNEVIE as due to the «lateral approximation of parallel filaments so that the split can no longer be observed». SHARP (1929) with different technique from that used by himself some years earlier finds two chromonemata in somatic telophase in *Vicia Faba*, *Tradescantia*, *Trillium*, *Allium* and *Podophyllum*; all these genera have large chromosomes. He considers that the chromonemata may remain close together or separate according to «conditions of growth, methods of fixation and other unknown local conditions». He believes, however, that the actual splitting of the chromonema takes place during prophase and that in plants with large chromosomes, the division of the chromonema is one complete mitotic cycle in advance of the division of the achromatic matrix.

In *Nicandra*, the achromatic body of the chromosome can be followed during telophase and interphase and only one spiral could be distinguished

running lengthwise without breaks, FIG. 2. In the somatic mitosis of *Nicandra* evidence of the split occurs only in the spireme of prophase, FIG. 4. It was impossible to determine whether or not the single spiral chromonema seen in resting nuclei is actually two parallel and closely associated threads.

In the early prophases of meiosis as the achromatic matrix gradually disappears and the chromonemata elongate, only a single zigzag thread was observed to correspond to each chromosome. As the archesporial cells prepare for meiosis the nuclear volume increases and the earlier zigzag appearance of the chromosomes is lost in a leptonema in which the threads are so fine that it is very difficult to say whether they are single or double. Reasoning from the observation on resting nuclei, the writer believes that the fine thread of leptonema is probably still single. It is not until later stages of prophase when the pachytène threads emerge from synizesis that duality is clearly apparent. Consequently it seems as though the split occurs during prophase of meiosis in microsporocytes. In *Nicandra* at late diakinesis and metaphase of the heterotypic division the chromosomes are very much condensed and the chromatids appear as straight rods as observed in *Secale* by SAX (1930). The spiral structure is apparently no longer present at metaphase which condition may facilitate the separation of the daughter chromosomes during early anaphase.

Method of Chromosome Pairing.

These observations on meiosis in *Nicandra* show pairing to take place by parasynapsis. A telosynaptic interpretation has been given in a number of the Solanaceae plants by workers who believed there was a so-called «continuous spireme» present in prophase. In *Nicandra* at early diplotène, FIG. 16, it is possible to follow some of the paired chromosomes throughout their length and even as the chromosomes recover from synizesis they seem to be already paired parasynaptically, FIG. 15. Terminal attachments and nodes or points of contact which correspond to the chiasmata described in *Tulipa* by NEWTON (1927) and in *Lilium* by BELLING (1928) can be distinguished during diakinesis and at metaphase. Pairing is by chiasmata or the interchange of partners among the paired chromatids, a condition which DARLINGTON (1929) has described as universal in meiosis.

The parallel association of homologous chromosomes in resting somatic cells, FIG. 1, confirms the parasynaptic interpretation.

Phylogenetic Relationship.

In *Nicandra* the 10 pairs of chromosomes are represented by at least 5 different lengths as seen from the study of bivalents during late prophase and metaphase stages. The chromosome complex may be represented by the formula $L_2 + l_2 + 4M_2 + 3m_2 + S_2$. This shows a striking similarity to that of *Datura* with 12 chromosomes which according to BELLING and BLAKESLEE (1923) is represented as $L_2 + 4l_2 + 3M_2 + 2m_2 + S_2 + s_2$. The second largest

pair in *Nicandra*, l_2 , seem to be absent from *Datura* where there is a long «*L*» pair and then a group next in size of four pairs. If the «*l*» chromosomes of *Nicandra* should fragment they might give rise to additional small chromosomes such as are found in *Datura*.

Further evidence in support of a close affinity between the chromosomes of *Datura* and *Nicandra* is found in the configuration of the bivalents. O'NEAL (1920) figures what may be taken as terminal and interstitial chiasmata at diakinesis in *Datura* as seen in *Nicandra*. His metaphase figures do not indicate the persistence of the nodes observed in diakinesis which persistence is present in at least three chromosomes of *Nicandra*. According to BELLING the bivalents of *Datura* are united at one or both ends at metaphase forming **V**'s and **O**'s a condition frequently present in *Nicandra*. BLAKESLEE (1929) reports the presence of a «hump» discovered by Dr BERGNER in one of the median chromosomes of *Datura*. Persisting subterminal interstitial chiasmata in *Nicandra* cause a small protuberance or hump on ring and **V** shaped bivalents in metaphase (see TEXT FIG. 5). This has been observed on three different bivalents in *Nicandra*. The presence of a «hump» (which must be taken as due to an interstitial chiasma) on only one bivalent in *Datura* suggests that the chromosomes of *Datura* may be shorter than those of *Nicandra* and therefore might have a lower chiasma frequency.

SUMMARY.

1. The chromosome count of *Nicandra physaloides* (L.) GAERTN. is $n = 10$, $2n = 20$, and no cytological difference could be found between *Nicandra physaloides* var. «immaculata» and *Nicandra physaloides* var. «typica».

2. The chromosomes in the resting nuclei of somatic cells undergo very little change. Homologous chromosomes are found associated in pairs.

3. There is a single spiral chromonema in each chromosome of a resting nucleus.

4. The chromosomes elongate during somatic prophase to form a loose system of spireme thread. The split observed at metaphase is initiated at this time or earlier.

5. The condensation of the coiled spireme threads gives rise to 20 split chromosomes.

6. The attachment of spindle fibres is median in all the chromosomes.

7. The last mitosis in the archesporial cells is similar to other sporophyte mitoses.

8. There is a long period of rest between the last somatic division and the beginning of prophasic activity in the microsporocytes. During this period the chromosomes pass into a network of delicate chromatic threads.

9. Synizesis is a constant feature of meiosis in *Nicandra* and may be due to osmotic disturbance in the nucleus.

10. The pachynema that emerges from synizesis is a longitudinally split thread in which the two halves are closely twisted about each other.

11. Pairing is first observed after synizesis. The mode of chromosome pairing is parasynaptic and followed by the formation of chiasmata.

12. The chromosomes of *Nicandra* are of at least 5 different lengths and may be expressed by the formula $[L_2 + l_2 + 4M_2 + 3m_2 + S_2]$.

13. There is a wide range of variation in the configuration of the bivalents during late heterotypic prophase. This is due to the different numbers and positions of the chiasmata by which the paired chromosomes are joined.

14. There is a reduction in the number of chiasmata between early diplotène and late diakinesis owing to their terminalisation. The chiasmata observed during late diakinesis persist during metaphase.

15. The chromosomes are very much condensed during metaphase and the spiral structure is not evident.

16. Nuclei are formed during interkinesis in which the chromosomes are distinguishable as split bodies.

17. The homoeotypic division is orderly. The two spindles may be in the same plane or at right angles.

18. Cytokinesis is effected by furrows that cut into the cytoplasm from the periphery.

19. The chromosomes in the nuclei of the tetrad are found to be split.

20. The chromosome organization in *Nicandra* resembles that of *Datura* suggesting a close phylogenetic relationship between the two genera.

LITERATURE CITED.

- 1880 Baranetzky, J. : Die Kerntheilung in der Pollenmutterzellen einiger Tradescantien; Bot. Zeit., **38**, 241-247, 265-274, 281-295.
- 1929 Babcock, E. B. & Clausen, J. : Meiosis in two species and three hybrids of *Crepis* and its bearing on taxonomic relationships; Univ. Calif. Pub. Agr. Sci., **2**, 401-432.
- 1913 Beer, R. : Studies in the spore development. III. The premeiotic and meiotic nuclear divisions of *Equisetum arvense*; Ann. Bot., **27**, 643-659.
- 1922 Belar, K. : Untersuchungen an *Actinophrys* sol. Ehrenberg; Arch. f. Prot., **46**, 1-96.
- 1923 Belling, J. & Blakeslee, A. F. : The reduction division in haploid, diploid, triploid and tetraploid daturas; Proc. Nat. Acad. Sci., **9**, 106-111.
- 1928 Belling, J. : Nodes and chiasmata in the bivalents of *Lilium* with regard to segmental interchange; Biol. Bul., **54**, 465-470.
- 1929 Blakeslee, A. F. : Cryptic types in *Datura*; Journ. Hered., **20**, 177-190.
- 1908 Bonnevie, K. : Chromosomenstudien. I. Chromosomen von *Ascaris*, *Allium* und *Amphiuma*; Arch. Zellf., **2**, 201-278.
- 1911 Id. : Id. III. Chromatinreifung in *Allium Cepa* ♂; Arch. Zellf., **6**, 190-253.
- 1925 Castetter, E. F. : Studies on the comparative cytology of the annual and biennial varieties of *Melilotus alba*; Amer. Jour. Bot., **12**, 270-286.
- 1926 Id. : Cytological studies in the Cucurbitaceae. I. Microsporangogenesis in *Cucurbita maxima*; Amer. Jour. Bot., **13**, 1-10.
- 1925 Campin, M. G. : A cytological study of pollen development in *Nolana*; New. Phytol., **24**, 17-23.
- 1927 Chipman, R. H. & Goodspeed, T. H. : Inheritance in *Nicotiana*. VIII. Cytological features of *Purpurea* haploid; Univ. Calif. Pub. Bot., **2**, 141-158.
- 1924 Dahlgren, O. K. V. : Kreuzungskleinigkeiten. Versuche mit *Capsella bursa pastoris*, *Lactuca muralis*, *Fagopyrum emarginatum*, *Geranium Robertianum*, *Geum rivale*, *Dracocephalum thymiflorum* und *Nicandra physaloides*; Hereditas, **5**, 222-230.
- 1929 Darlington, C. D. : Ring formation in *Oenothera* and other genera; Jour. Gen., **20**, 345-363.
- 1929 Id. : Meiosis in Polyploids. II. Aneuploid *Hyacinthus*; Jour. Gen., **21**, 17-56.
- 1929 Id. : Chromosome Organisation and Structural Hybridity in the Tradescantiae; Jour. Gen., **21**.

- 1916 Farr, C. H. : Cytokinesis in the pollen mother cells of certain dicotyledons; Mem. New-York Bot. Gard., **6**, 253-317.
- 1918 Id. : Cell division by furrowing in *Magnolia*; Amer. Jour. Bot., **5**, 379-395.
- 1922a Id. : Meiotic cytokinesis of *Nelumbo*; Amer. Jour. Bot., **9**, 296-306.
- 1922b Id. : Quadripartition by furrowing in *Sisyrinchium*; Bull. Torr. Bot. Club, **49**, 51-62.
- 1920 Farr, W. K. : Cell division of the pollen mother cell of *Cobaea scandens alba*; Bull. Torr. Bot. Club, **47**, 325-338.
- 1927 Gates, R. R. : Observation on the pollen development of two species of *Lathraea*; Jour. R. Micros. Soc., **47**, 209-225.
- 1923 Goodspeed, T. H. : A preliminary note on the cytology of *Nicotiana* species and hybrids; Svensk. Bot. Tid., **17**, 472-478.
- 1928 Heimlich, L. F. : Microsporogenesis in *Cucumis sativus*; La Cellule, **39**, 7-24.
- 1924 Karpechenko, C. D. : Hybrids of *Raphanus sativus* $\times$ *Brassica oleracea*; Jour. Genet., **14**, 375-396.
- 1930 Kato, L. : Cytological studies of pollen mother cells of *Rhoeo discolor* Hance,; Mem. Coll. Sci. Kyoto, **5**, 139-161.
- 1925 Kaufmann, B. P. : The existence of double spiral chromatin band and a « bouquet stage » in *Tradescantia pilosa* Lehm.; Amer. Nat. **59**, 190.
- 1926 Id. : Chromosome structure and its relations to the chromosome Cycle. I. Somatic mitosis in *Tradescantia pilosa*. II. *Podophyllum peltatum*; Amer. Jour. Bot., **13**, 59-80, 355-363.
- 1926 Kuwada, Y. : On the structure of the anaphasic chromosomes in the somatic mitosis in *Vicia Faba*,; Mem. Coll. Sci. Kyoto Imp. Univ., B, **2**, No. 1.
- 1927 Id. : On the spiral structure of chromosomes; Bot. Mag. Tokyo, **41**, 100-109.
- 1929 Kulkarni, C. G. : Meiosis in pollen mother cells of strains of *Oenothera pratincola* Bartlett; Bot. Gaz., **87**, 218-258.
- 1907 Laibach, T. : Zur Frage nach der Individualität der Chromosomen im Pflanzenreich; Beih. Bot. Centralbl., **22**, 191-210.
- 1926 Lesley, M. M. : Maturation in diploid and triploid tomatoes; Genetics, **9**, 260-279.
- 1921 de Litardière, R. : Recherches sur l'élément chromosomique dans la caryocinèse somatique des Filicinées; La Cellule, **31**, 255-473.
- 1909 Lundegårdh, H. : Ueber Reduktionsteilung in den Pollenmutterzellen einiger dicotylen Pflanzen; Svensk. Bot. Tidsk, **3**, 78-124.
- 1928 Maeda, T. : On the spiral structure of chromosomes in sweet pea (*Lathyrus odoratus*); Bot. Mag. Tokyo, **42**, n° 496, 191-195.

- 1930 Maeda, T. : The meiotic divisions in pollen mother cells of the sweet pea (*Lathyrus odoratus*),; Mem. Coll. Sci. Kyoto Imp. Univ., **5**, 89-123.
- 1929 McClintock, B. : A method of making aceto-carminic smears permanent; Stain Tech., **4**, 53-56.
- 1922 Martens, P. : Le cycle du chromosome somatique dans les Phanérogames. I. *Paris quadrifolia*; La Cellule, **32**, 333-428.
- 1925 Id. : Id. II. *Listera ovata*; La Cellule, **36**, 125-214.
- 1905 Miyake, K. : Ueber Reduktionsteilung in den Pollenmutterzellen einiger Monokotylen; Jahrb. wiss. Bot., **42**, 83-120.
- 1927 Newton, W. C. F. : Chromosome studies in *Tulipa* and some related genera; Jour. Linn. Soc. Bot., **47**, 339-354.
- 1929 Newton, W. C. F. & Darlington, C. D. : Meiosis in polyploids. I. Triploid and pentaploid tulips; Genetics, **21**, 1-15.
- 1920 O'Neal, C. E. : Microsporogenesis in *Datura stramonium*; Bull. Tor. Bot. Club, **47**, 231-241.
- 1906 Overton, J. B. : Ueber Reduktionsteilung in den Pollenmutterzellen einiger Dikotylen; Jahrb. wiss. Bot., **42**, 121-153.
- 1909 Id. : On the organization of the nuclei in the pollen mother cells of certain plants; Ann. Bot., **23**, 19-61.
- 1904a Rosenberg, C. : Ueber die Tetradenteilung eines *Drosera*-Bastardes; Ber. Bot. Ges., **22**, 47-53.
- 1904b Id. : Ueber die Individualität der Chromosomen im Pflanzenreich; Flora, **93**, 251-259.
- 1907 Id. : Zur Kenntnis der presynaptischen Entwicklungsphasen der Reduktionsteilung; Svensk. Bot. Tids., **1**, 398-410.
- 1909 Id. : Zur Kenntnis von den Tetradenteilungen der Compositen; Svensk. Bot. Tidsk., **3**, 64-77.
- 1897 Sargent, E. : The formation of sexual nuclei in *Lilium Martagon*. II. Spermatogenesis; Ann. Bot., **11**, 187-224.
- 1930 Sax, K. : Chromosome structure and mechanism of crossing over; Jour. Arnold Arbor., **11**, 193-220.
- 1909 Schaffner, J. H. : The reduction division in the microsporocytes of *Agave virginica*; Bot. Gaz., **47**, 198-214.
- 1929 Sharp, L. W. : Structure of large somatic chromosomes; Bot. Gaz., **88**, 349-382.
- 1930 Shinke, N. : On the spiral structure of chromosomes in some higher plants; Mem. Coll. Sci. Kyoto Imp. Univ., **5**, 239-245.
- 1907 Strasburger, E. : Ueber die Individualität der Chromosomen und die Propfhybriden-Frage; Jahrb. wiss. Bot., **44**, 482-555.
- 1922 Taylor, W. R. : Organization of heterotypic chromosomes; Science, **56**, 635.
- 1924 Id. : The smear method of plant cytology; Bot. Gaz., **78**, 236-238.

- 1927 Vilmorin, R. & Simonet, M. : Recherches sur le nombre des chromosomes des Solanées; Verh. V. Int. Kongr. f. Vererb., **2**, 1520-1536.
- 1926 Weinstein, A. I. : Cytology of *Phaseolus vulgaris*; Amer. Jour. Bot., **13**, 248-263.
- 1929 Weier, T. E. : A comparison of meiotic prophase in *Oenothera Lamarckiana* and *Oenothera Hookeri*; La Cellule, **39**, 271-302.
- 1906 Yamanouchi, S. : The life history of *Polysiphonia violacea*; Bot. Gaz., **42**, 401-449.
-

EXPLANATION OF FIGURES.

All figures were drawn with the aid of a camera lucida using ZEISS compensating ocular $\times 20$ and apochromatic objective 1,5, N. A. 1,30. Approximate magnification 3500 diam. (Reduced to 3/4 of the original size in reproduction.)

FIG. 1. Resting somatic nucleus from anther, 20 chromosomes associated in pairs. (a) one pair longer than the others.

FIG. 2. Resting somatic nucleus from anther. Each chromosome with a single spiral chromonema.

FIG. 3. Somatic nuclei from nucellus with the chromosomes elongating at the approach of mitosis.

FIG. 4. The same stage as FIG. 5 from a root-tip showing the split thread.

FIG. 5. A somatic nucleus before metaphase with split chromosomes.

FIG. 6. Somatic metaphase.

FIG. 7. The somatic metaphase plate viewed from the pole of the spindle.

FIG. 8. Early anaphase of somatic mitosis.

FIG. 9. The formation of daughter somatic nuclei and the association of daughter chromosomes in pairs.

FIG. 10. Daughter nuclei following the last archesporial mitosis with the chromosomes associated in pairs. (a) longest.

FIGS. 11 and 12. Later stages in the microsporocyte with the chromosomes very much elongated.

FIG. 13. The microsporocyte with the nucleus at the end of the resting condition; network of fine threads.

FIG. 14. Microsporocyte with nucleus entering synizesis showing the contraction of threads away from the nuclear membrane.

FIG. 15. Recovery from synizesis.

FIG. 16. Pachytène thread; evidence of pairing appears.

FIG. 17. Early diakinesis.

FIG. 18. Diakinesis showing 10 bivalents. L_2 the longest pair of chromosomes.

FIG. 19. Late diakinesis. The nuclear membrane breaking down and the nucleolus disorganized.

FIG. 20. Bivalents grouped before the formation of the heterotypic spindle.

FIG. 21. Early metaphase with multipolar spindle.

FIG. 22. Bipolar heterotypic spindle.

FIG. 23. Late heterotypic anaphase.

FIG. 24. Polar views of heterotypic anaphase showing 10 split chromosomes.

FIG. 25. Formation of daughter nuclei with split chromosomes.

FIG. 26. The assemblage of split chromosomes for the homeotypic division; a few chromosomes joined to one another by delicate fibres.

FIG. 27. Homoeotypic metaphase. Bipolar spindle.

FIG. 28. Early homoeotypic anaphases; in this case a parallel arrangement of spindles.

FIG. 29. Late homoeotypic anaphases showing a few lagging chromosomes.

FIG. 30. Formation of the microspore nuclei; 10 split chromosomes evident.

FIG. 31. Microsporocyte showing the beginning of cytokinesis by furrowing.

TABLE OF CONTENTS.

Introduction	89
Material and methods	89
I. The organization of chromosomes in the resting nuclei of somatic cells	91
II. Somatic mitosis	92
III. The resting nuclei of the microsporocytes	93
IV. Meiosis in the microsporocytes	94
1. Heterotypic Prophase	94
a) Presynizesis	94
b) Synizesis	94
c) Pachytène	95
d) Diplotène and Diakinesis	95
2. Heterotypic Metaphase	96
3. Heterotypic Anaphase and Telophase	96
4. Interkinesis	96
5. Homocotypic Division	97
6. Cytokinesis	97
V. The configuration of bivalents in the heterotypic division	98
VI. Discussion	101
Summary	103
Literature cited	105
Explanation of figures	109

